

Original Article

Plastral shape isometry in Western Hermann's tortoise (*Testudo hermanni hermanni*)

Pere M. Parés-Casanova* and Héctor Miralles

Department of Animal Production, University of Lleida Av. Alcalde Rovira Roure 191, 25198-Lleida (Catalonia, Spain)

*Corresponding Author

Pere M. Parés-Casanova,
Department of Animal Production,
University of Lleida Av. Alcalde Rovira Roure 191,
25198-Lleida (Catalonia, Spain)
E-mail: peremiquelp@prodan.udl.cat

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Abstract

Elliptical Fourier Analysis is a good technique for characterizing the shape of complex biological and non-biological morphologies. The current investigation aimed to study plastral pigmentation contour (black areas of the ventral shell) changes, according to body size (plastral length), in Western Hermann's tortoise (*Testudo hermanni hermanni*), using Elliptical Fourier Analysis. For this goal, 52 domestic pure tortoises from authorized private breeders were selected, ventral pictures were individually taken and their contour automatically digitized and straight-line plastron length obtained. Straight-line plastron length ranged from 83.6 to 150.6 mm (for males) and from 78.9 to 171.8 mm (for females). A regression was performed for second and third harmonics as dependent variables, against plastral length (log transformed) as independent variable. Based on this sample, it is demonstrated that plastral pigmentation design does not change along animals' life-history. Therefore, a plastral pigmentation shape isometry can be supposed, at least for this subspecies.

1. Introduction

Hermann's tortoise (*Testudo hermanni*) is one of the four tortoise species classically placed in the genus *Testudo*. The others species are the marginated tortoise (*T. marginata*), Greek tortoise (*T. graeca*) and Russian tortoise (*T. horsfieldii*). *T. hermanni* is widely distributed in Western and Southern Europe, from Catalonia up to European Turkey [1]. Most populations in the Western part have been severely reduced the last decades, whilst the species appear still abundant in Eastern areas.

T. hermanni is a small tortoise species (maximum shell length approximately 22 cm). It has an intensive coloration, its yellow coloration making a strong contrast to the dark patches [2]. These black colours wash out in older animals, but the intense yellow is often maintained. The male tail is larger than in females and possesses a spike.

Two subspecies of *Testudo hermanni* are currently recognized: *T. hermanni hermanni* and *T. h. boettgeri* [3]. *T. h. hermanni* inhabits the Western part of its distribution range (Spain, France and Central and Southern Italy) and *T. h. boettgeri* inhabits the Eastern part of the range (North Eastern Italy, Balkan regions, Greece and Turkey) respectively. Since the 2000s, *T. h. hermanni* has been intensely studied, focusing on genetics, which has shown a clear genetic separation between *hermanni* and *boettgeri* [4]. *T. h. hermanni* includes the former *T. h. robertmertensi* (the name of a morph with very prominent cheek spots) and a number of local forms. In spite of genetic studies, it is clear that the analysis of plastron morphology represents a cheap and efficient tool for the study of *Testudo hermanni*. Nevertheless, can we be sure that this design does not change with growth?

Size, shape, and their relationship are important topics in organismal biology, and varied and efficient methods have been developed to describe natural objects. Conventional metrical morphometrics, consisting of areas, distances, angles and ratios, allows information about size, whereas geometric morphometrics retains all geometric information (size but also shape). The shape of the plastral pigmentation (i.e. the black areas of the ventral shell) design of *Testudo* is an absolutely irregular curved structure, where the conventional metrical approach is poorly suited. Procrustes-type landmarks, typical of geometric morphometrics, are difficult to be applied effectively, too. But the plastral pigmentation morphology can be quantified using an alternative geometric procedure, Elliptic Fourier Analysis (EFA). EFA, developed by Kuhl and Giardina [1982][5], collects outline data automatically from images and computes and visualizes shape changes,

transforming the data from a spatial domain into a frequency domain. Moreover it permits the re-creation, at any time, of the shape.

EFA has been successfully applied to a number of morphological structures [6]. As EFA is ideally suited to characterize the shape of complex morphologies [5], this research aimed to study plastral pigmentation contour changes, according to body size, in Western Hermann's tortoise (*Testudo hermanni hermanni*), using EFA. To the authors' knowledge, nothing similar has been applied to the study of this species.

2. Materials and methods

2.1 Sampling

A sample of 52 domestic pure tortoises belonging to authorized private breeders was studied. From them, 38 animals (16 males and 22 females) were descendants of native animals from Balearic Islands and the 14 (6 males and 8 females) from a continental Iberian area (Albera, NE Iberian Peninsula), but no geographical distinction was done. Selected animals were sexed and manually measured (i.e. straight-line plastron length, from the front of the gular scute to the rear of the plastron) [7]. Since, in our study, we used tortoises randomly collected from different owners, we could not detail life-history variables for all specimens. Although the number of scute annuli has been proposed as a reliable method for the aging of *Testudo* species, it is only highly accurate for the aging of juveniles and subadults [8], and its precision is criticized by some authors [9]. This is why we did not consider "age" but rather plastron length as the independent variable.

2.2 Extraction of the outlines

Ventral image captures were performed with a Nikon (Tokyo, Japan) D70 digital camera (image resolution of 2,240 x 1,488 pixels) equipped with a Nikon AF Nikkor® 28-80 mm telephoto lens. The focal axis of the camera was parallel to the horizontal plane of reference and centred on the plastron (ventral shell) on each animal. Images included a scale (10 mm x 20 mm). As plastrum is very flat, one does not expect important distortions and a good reliability was expected. The photographs were directly input to computer as GIF files and, subsequently, image noise was manually removed and contour extracted using a graphics software package (Adobe Photoshop version 14.2.1.CC®). Finally, images were transformed to BMP-256 colour files. To avoid redundant information in symmetric structures, only the outline of each left pattern was studied.

2.3 Elliptical Fourier Analysis

Image capture was carried out using the SHAPE® software package developed by Iwata and Ukai (2002)[10], which identifies the outline of the pattern and generates an elliptic Fourier description. Briefly, the procedure is as follows: images are binarized (i.e. transformed into white for the bone outline and black for the background, in pixels), thus automatically obtaining and digitalizing the outlines of each continuous contour (interface between the black and the white pixels). In this study, an average of 3,290 points was positioned along the outline of each specimen. Shape was approximated by the first 20 harmonics (H_1 to H_{20}), each harmonic being characterized by four coefficients (Fourier Descriptors, FDs). FDs come from the sine and cosine part of the variation in the x and y coordinates [11] and define the ellipse on the xy -plane. The FDs are then normalized to be invariant of size, location, rotation, and starting position, according to Rohlf and Archie (1984)[12]. As SHAPE® adjusts for size and orientation, first harmonic (H_1) does not contain morphological information [13]. Therefore only seventy-six $[(4 \times 20) - 4]$ standardized FDs were considered for initial analysis. Straight-line plastron length was measured directly with a tape and expressed in mm.

2.4 Allometry

The relationship between shape variables and straight-line plastron length was evaluated by means of a multivariate linear regression analysis, using log transformed length data as independent

variable, and descriptors of second and third harmonics as dependent ones. In order to reduce FDs, a Principal Component Analysis for harmonics H_2 to H_{20} was performed from var-covar matrix. Only PC with eigenvalues values below Jolliffe cut-off were considered.

The statistical treatment was performed with the PAST® package[14]. The significance level was established at 5%.

3. Results

3.1 Differences between sexes

Straight-line plastron length ranged 83.6-150.6 mm (males) and 78.9-171.8 mm (females). Preliminary analyses revealed no significant differences between male and female shapes, ($p=0.183$) and data were thus pooled for further analyses.

3.2 Principal Component Analysis

Nine first principal components (PCs) with eigenvalues below jolliffe cut-off (3.481×10^{-10}) explained 89.0 % of the total variance observed (Table 1) but, due to the sampling limitations, the Fourier series was truncated at 8 PCs, the level at which the average cumulative power was 87.3%.

3.3 Regression on straight-line plastron length

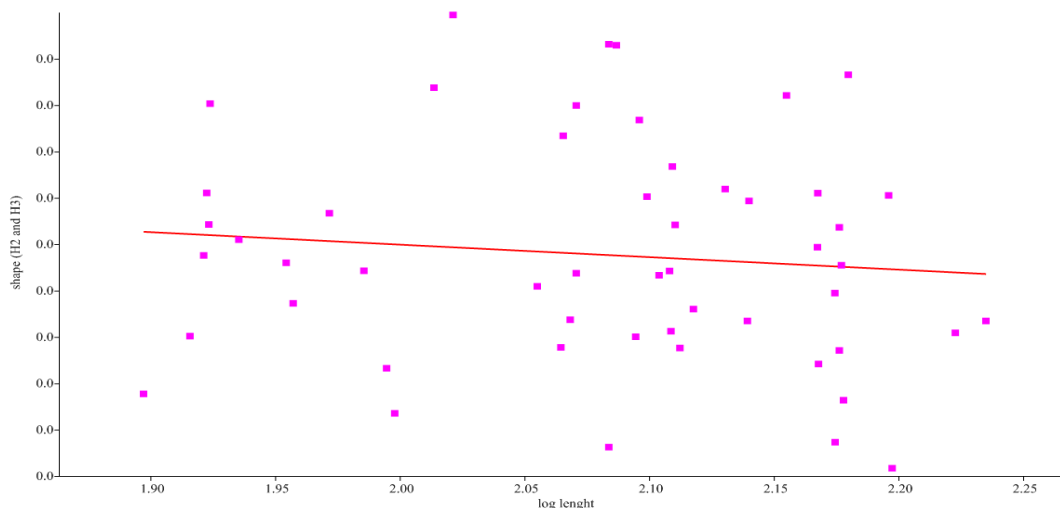
Regression was performed for FDs belonging to 8 first PCs. The regression of shape on straight-line plastron length ($123.0\text{mm} \pm 24.95$, range 78.9-171.7mm) was not significant ($R^2=0.134$, Wilk's $\lambda=0.465$, $p < 0.000001$) (Figure 1).

Table 1: Eigenvalues and variance explained for the first principal components (PC).

PC	Eigenvalue	% explained variance	% cumulative variance
1	1.35×10^{12}	53.377	53.377
2	2.47×10^{11}	9.734	63.111
3	1.58×10^{11}	6.217	69.328
4	1.49×10^{11}	5.865	75.193
5	1.02×10^{11}	4.022	79.215
6	8.83×10^{10}	3.482	82.698
7	6.38×10^{10}	2.514	85.213
8	5.22×10^{10}	2.058	87.272
9	4.45×10^{10}	1.754	89.026

Analysis was done from var-covar matrix using harmonics H_2 to H_{20} . Nine first principal components (PCs) with eigenvalues below Jolliffe cut-off (3.481×10^{-10}) explained 89.0 % of the total variance observed (Table 1).

Figure 1: Linear regression between log transformed straight-line plastron length and log transformed scores for FDs belonging to 8 first PCs. Regression showed non-significance ($R^2=0.134$, Wilk's $\lambda=0.465$, $p < 0.000001$).



4. Discussion

The results obtained showed no allometric relationship. Therefore, an isometry may be supposed. Isometric (*iso*=same, *metric*=measure) scaling describes the condition where objects of different sizes share the same shape. In this case, no plastral pigmentation pattern change (no shape change) can be supposed along life and/or corporal condition of animals (expressed as plastral length).

5. Conclusions

The presented results are considered to be encouraging and suggest the need for the initiation of further research to describe other geometrical descriptors of the plastral pigmentation pattern in tortoises. In the case of this study, it seems that typology of plastral shape of *T. hermanni* is irrespective of animal age and/or corporal condition.

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Conflicts of interest statement

The authors declare that there are no conflicts of interests.

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